

June 12, 1879.

The Annual Meeting for the election of Fellows was held this day.

THE PRESIDENT in the Chair.

The Statutes relating to the election of Fellows having been read, Dr. T. Graham Balfour and Mr. W. C. Roberts were, with the consent of the Society, nominated Scrutators to assist the Secretaries in examining the lists.

The votes of the Fellows present having been collected, the following candidates were declared duly elected into the Society :—

Prof. John Anderson, M.D.	Prof. Francis Stephen Bennet
Rev. Miles Joseph Berkeley,	François de Chaumont, M.D.
F.L.S.	Prof. George Downing Liveing,
Sir Henry Bessemer.	M.A.
Prof. Alexander Crum Brown,	George Matthey, F.C.S.
M.D.	George John Romanes, M.A.,
Walter Lawry Buller, Sc.D.,	F.L.S.
F.L.S.	Arthur Schuster, Ph.D., F.R.A.S.
George Howard Darwin, M.A.	Prof. Harry Govier Seeley, F.L.S.
Prof. Joseph D. Everett, M.A.,	Benjamin Williamson, M.A.
D.C.L.	Thomas Wright, M.D., F.G.S.

Thanks were given to the Scrutators.

June 19, 1879.

THE PRESIDENT in the Chair.

The Presents received were laid on the table, and thanks ordered for them.

The Rev. Miles Joseph Berkeley, Sir Henry Bessemer, Prof. Alexander Crum Brown, Mr. George Howard Darwin, Prof. Francis Stephen Bennet François de Chaumont, Prof. George Downing Liveing, Mr. George John Romanes, Dr. Arthur Schuster, Prof. Harry Govier Seeley, Mr. Benjamin Williamson, and Dr. Thomas Wright were admitted into the Society.

The following Papers were read :—

I. "On the Existence of Liebreich's Protagon in the Brain."

By Professor ARTHUR GAMGEE, M.D., F.R.S., Owens College, Manchester, and ERNST BLANKENHORN, Ph.D. Received April 29, 1879.

In the year 1865, Dr. Oscar Liebreich published an important paper* upon a new proximate principle which he had separated from the brain. Unlike the numerous bodies, possessed of ill-defined properties which had, by different writers, received the names of cerebrin, cerebrie acid, lecithine, or phosphorized fats, this new body could be extracted by an easy process in a state of purity, and to it, probably as indicating it as the first definitively ascertained specific constituent of brain, Liebreich ascribed the name of Protagon (*πρωταγος*, leading in advance).

The substance was obtained by the following process. An animal was bled to death from the carotid, and a stream of water was passed through the vessels of the head, so as to wash the blood out. The brain, freed from its membranes, was then pounded in a mortar and shaken in a flask with ether and water at 0° C. It was allowed to stand at a temperature of 0°, until the ether had separated, and the treatment with ether again and again repeated.

The brain matter having been separated by filtration from ether and water, was digested with 85 per cent. alcohol, at a temperature of 45° C. The fluid was filtered hot and allowed to cool at 0° C. A flocculent precipitate then separated, which was collected on a filter and treated with cold ether, until it ceased to dissolve cholesterin. The insoluble mass was dried *in vacuo*, and dissolved in spirit at 45° C. The alcoholic solution was filtered and allowed to cool *very gradually*, when protagon separated in the form of microscopical needles, differing a little in arrangement and form according to the concentration of the solution. The substance thus obtained could be recrystallized repeatedly. As a result of his analyses Liebreich ascribed to protagon the formula $C_{116}H_{241}NO_{22}P$.

The following exhibits the percentage composition of protagon according to this formula and the mean of all Liebreich's analyses:—

	Calculated for $C_{116}H_{241}N_4O_{22}P$.	Found.
C	67·21	66·74
H	11·59	11·74
N	2·70	2·80
P	1·50	1·23
O	17·00	17·49

* Oscar Liebreich, "Ueber die Chemische Beschaffenheit der Gehirns substanz," "Annalen der Chemie und Pharmacie," Bd. cxxxiv, s. 29–44.

Protagon is soluble with difficulty in cold, but more easily in warm alcohol and ether. At higher temperatures than 55° C., alcohol appears to decompose protagon. In water protagon swells and presents the appearance of an opaque jelly, ultimately dissolving so as to form an opaque solution. Liebreich found that protagon was soluble in glacial acetic acid, which deposited it again in a crystalline form, when subjected to the action of cold.

When boiled with a solution of barium hydrate protagon is decomposed, yielding glycerin—phosphoric acid, fatty acids of which he isolated stearic acid in a state of purity, and a base to which he gave the name of *neurine*, and to the platinum compound of which he ascribed the formula $C_5H_{14}NCl_3Pt$. This base was afterwards shown to be identical with the base which Strecker had separated from bile and termed *choline*.

Although the absolute accuracy of a large number of Liebreich's facts has been placed beyond question, the cardinal fact itself—that protagon is a definite phosphorized principle contained in nervous matter—has come to be universally denied.

By Diaconow, Hoppe-Seyler, and Thudichum it is denied that any such definite substance exists, and Liebreich's protagon is held to be a mechanical admixture of a phosphorized body termed *lecithin*, $C_{44}H_{90}NPO_9$, with a nitrogenous, non-phosphorized, body termed *cerebrin*. The presence of phosphorus in protagon is said to be due to contamination with lecithin, and in support of this view it is alleged that by extracting protagon with ether, the substance loses more and more phosphorus; according to Diaconow and Hoppe-Seyler's admission, the phosphorus does adhere most obstinately and cannot be entirely got rid of, though Dr. Thudichum thinks he has, by mere extraction with ether, obtained cerebrin (or cerebrines) quite free from phosphorus.

It is not our intention in this communication to enter into a full criticism of the views of those who have preceded us in the investigation of the existence or non-existence of protagon, and we shall confine ourselves to a statement of a few of the chief objections which appeared to us as insurmountable obstacles in the way of accepting Diaconow's and Hoppe-Seyler's statements.

Liebreich's protagon was described by its author as a white non-hygroscopic substance, which could, by suitable treatment, be constantly obtained in a crystalline form, and this description will be shown in the sequel to be absolutely correct.

Cerebrin is a body most imperfectly known, obtained by its discoverer, Müller,* by pounding up brains with baryta water to the con-

* Dr. Wilhelm Müller. "Ueber die Chemischen Bestandtheile des Gehirns-zweite Abhandlung." "Annalen der Chemie und Pharmacie." Bd. cv (1858), s. 361.

sistence of a thin milk and then boiling; a precipitate forms, which is extracted with boiling alcohol. On cooling, the fluid deposits an abundant precipitate; this is treated with ether, to separate cholesterol and fats, and then recrystallized from boiling alcohol. Of this body Müller published two analyses, which led him to assign to it the formula $C_{34}H_{33}NO_6$. It is described by Hoppe-Seyler as a light, white, *highly hygroscopic* powder.*

But what right have we to assume that a body obtained by boiling so complex an organic mixture as a brain with caustic baryta is a proximate principle of the brain, *i.e.*, that it exists preformed in the brain? The surmise is as bold as it is improbable.

Lecithin is described by Diaconow himself as a non-pulverizable, yellowish-white, *highly hygroscopic* body.† Is it not strange that two usually amorphous highly hygroscopic bodies should, when mixed, give rise to a constantly crystallizable absolutely non-hygroscopic mixture? And this is exactly what would be the case were the hypotheses of Diaconow and Hoppe-Seyler correct.

According to Diaconow, by repeated extractions with ether, the P contained in protagon may be made to sink to 1 per cent., whereas, according to Liebreich's formula, it should contain 1.5 per cent. Actually, in the three determinations which he made, Liebreich obtained 1.1, 1.1, and 1.5, as the amount of phosphorus in 100 parts, but, unfortunately, he seems to have concluded that the highest number was correct and made it the basis of his calculation.

In the year 1877, one of us, assisted by Mr. Leopold Larmuth, Platt Physiological Scholar, commenced in the Physiological Laboratory of Owens College, a series of experiments intended to determine whether Liebreich's protagon existed or not. This preliminary investigation showed that by Liebreich's process there is always obtained a body having the physical properties of protagon, and containing phosphorus in a proportion sufficiently near to that indicated by him; it was found that the amount of phosphorus in specimens of protagon which had been crystallized from alcohol four or five times, was not smaller than that present in protagon which had only once been crystallized, though a thorough treatment with ether preceded each recrystallization.

These first experiments so far as they went, were perfectly satisfactory. It appeared, however, quite essential, before forming a definite opinion, to extend them very considerably and especially to

* "Auf diese Weise dargestellt bildet das Cerebrin ein leichtes weisses sehr hygroscopisches Pulver," &c. Hoppe-Seyler, "Handbuch der Physiologisch—und Pathologisch—Chemischen Analyse." Dritte Aufl. S. 169.

† "Das reine Lecithin stellt eine gelblich-weiße, wachsartige, in dünner Schicht ausgebreitet seiden-glänzende, sehr hygroscopische Masse dar." Diaconow, "Ueber die Chemische Constitution des Lecithin." "Centralblatt f. d. Med. Wissenschaften," 1868, p. 2.

prove the definite nature of protagon by a large number of analyses, indicating not merely the amount of phosphorus, but also that of the other elements present in it.

This investigation we have lately been able to carry out with all necessary minuteness, and having, to our entire satisfaction, established the real existence of protagon, we communicate the results obtained before proceeding to a fresh study of the products of decomposition of that body.

Process employed in the preparation of Protagon in the present research.

At first we commenced by repeating exactly the process of Liebreich in all its details; one of the steps of that process we had found fraught with peculiar difficulty, and we soon ascertained that it could be dispensed with without prejudicially affecting the success of the operations. The step to which we refer consists in agitating the freshly pounded brain repeatedly with water and ether at 0° C., so long as the ether dissolves considerable quantities of substance, then filtering and placing the insoluble matter into 85 per cent. alcohol at 45° C. When pounded brain is so treated with water and ether it swells up and the separation of the ether is most incomplete. The process of filtration is one which is attended with great difficulty, even when carried out in the only way in which we found it possible, viz., in the woven bags sold for household purposes for straining jellies, &c. By the kindness of Professor Osborne Reynolds, F.R.S., a small model of a centrifugal hydro-extractor, similar to that used in sugar refineries, was constructed for us in the engineering workshop of the Owens College, and was driven by the laboratory steam-engine, and this was for some time efficiently employed in separating the portion of the brain insoluble in water and ether from these fluids. It was, however, obvious that however prolonged the ether washing, it never succeeded in freeing the brain from cholesterin and other matters soluble in ether, and that the removal of these bodies from protagon was most readily effected at a later stage of the operations. As, ultimately modified, our process was as follows:—

Perfectly fresh ox's brains were freed from blood and from adhering membranes as completely as possible, and were then digested for many hours (18 to 24) in 85 per cent. alcohol in a large incubator kept constantly at 45° C. The fluid was filtered whilst hot, and the insoluble matter was again treated with fresh quantities of spirit, the proceeding being repeated four or five times, as long, indeed, as the filtrate when cooled to 0° deposited a fair quantity of white flocculent precipitate. This precipitate was collected on a filter, and being then transferred to a stoppered bottle was thoroughly and repeatedly agitated with ether, in order to dissolve cholesterin and other bodies soluble in ether. The ether having been removed, first by decantation

and then by filtration, the substance left undissolved by it was first of all dried between sheets of filtering paper in air, and afterwards over sulphuric acid or phosphorus pentoxide. The resulting mass, which had a snow-white colour, was reduced to powder, moistened with a little water, and digested for many hours with alcohol heated to 45° C. From the filtered liquid, if this be allowed to cool *very gradually*, the proton separates in the form of microscopic crystals, mostly arranged in rosettes, the appearance and arrangement of which differs somewhat, as Liebreich very exactly pointed out, according to the degree of concentration of the solution. The once crystallized proton thus obtained was collected on a filter, washed with ether, and dried first of all in air and ultimately over P_2O_5 . It was then recrystallized as many times as required, the process always commencing by pulverizing and thoroughly shaking with cold ether.

It appeared to us of special importance not merely to subject to ultimate analysis several different samples of proton, but specially to subject to ultimate analysis proton after the second crystallization, and the same proton after being recrystallized a third time.

In our analyses the C and H were determined by combustions made with lead chromate, with an abundance of reduced copper in the fore part of the combustion tube. The N determinations were made by Dumas' method, the phosphorus was determined in some cases by Carius's method, in others by fusion with sodium carbonate and saltpetre. The results of every combustion, save two, in which unavoidable accidents occurred, are given below.

The following are the results of the analyses:—

1. *Proton twice recrystallized from ox brain.*
 0.288 grm. gave 0.702 CO_2 and 0.284 H_2O .
 0.355 ,, 7 cub. centims. of N (at 0° C. and 760 millims.).
 0.296 (treated by Carius's method) gave 0.0116 grm. of $Mg_2P_2O_7$.
2. *Twice recrystallized proton from ox brain (same sample as 1).*
 0.306 grm. gave 0.747 CO_2 and 0.2955 H_2O .
 0.262 ,, 6 cub. centims. of N (at 0° C. and 760 millims.).
 0.3025 (treated by Carius's method) gave 0.012 grm. of $Mg_2P_2O_7$.
3. *Twice crystallized proton from ox brain (different sample from 1 and 2).*
 0.284 grm. gave 0.691 grm. of CO_2 and 0.270 grm. of H_2O .
 0.388 ,, 8 cub. centims. of N at 0° C. and 760 millims.
 0.782 ,, 0.0291 grm. of $Mg_2P_2O_7$.
4. *Twice crystallized proton from ox brain (same sample as 3).*
 0.2968 grm. gave 0.722 CO_2 and 0.288 grm. of H_2O .
 0.5368 ,, 0.02 grm. of $Mg_2P_2O_7$.

5. *Once crystallized protagon from dog's brain.*

0·305 grm. gave 0·7415 grm. of CO_2 and 0·289 grm. of H_2O . Neither P nor N determined.

6. *Once crystallized protagon from dog's brain* (same sample as 5).

0·25 grm. gave 0·6103 grm. of CO_2 and 0·249 grm. H_2O .

7. *Four times crystallized protagon* (prepared in 1877 by Mr. Lar-muth).

0·214 grm. gave 0·5201 grm. CO_2 and 0·202 grm. H_2O .

8. *Three times crystallized protagon* (prepared by recrystallizing the body which had furnished the samples used in Analyses 3 and 4).

0·214 grm. gave 0·5203 grm. CO_2 and 0·2015 grm. of H_2O .

0·48 „ 9·7 cub. centims. of N (at 0°C . and 760 millims.).

0·5164 „ 0·019 grm. of $\text{Mg}_2\text{P}_2\text{O}_7$.

The results are shown in a tabular form below:—

	Protagon once recrystallized (dog).		Protagon twice recrystallized (ox).		Protagon twice recrystallized (ox).		Thrice re- crystallized (ox).	Four times recrystallized (horse).
	No. 5.	No. 6.	No. 1.	No. 2.	No. 3.	No. 4.	No. 8.	No. 7.
C. . .	66·3	66·6	66·46	66·58	66·34	66·35	66·30	66·26
H. . .	10·52	11·06	10·96	10·72	10·56	10·78	10·467	10·48
N. . .	..	..	2·3	2·6	2·40	..	2·29	..
P. . .	..	..	1·094	1·107	1·032	1·081	1·027	..
O.								

From the above numbers we have deduced for protagon the empirical formula $\text{C}_{160}\text{H}_{308}\text{N}_5\text{PO}_{35}$.

	Calculated.		Mean found.	
C_{160}	1920		66·45	 66·39
H_{308}	308		10·66	 10·69
N_5	70		2·42	 2·39
P.	31		1·07	 1·068
O_{35}	560		19·40	
	2889		100·00	

A study of our results leaves no doubt of the definite and, within certain limits, stable character of protagon. It is to be borne in mind that in no case were the solutions containing protagon exposed to a higher temperature than 45°C ., as we have good reason to believe that Liebreich was right in asserting that at higher temperatures protagon undergoes decomposition.